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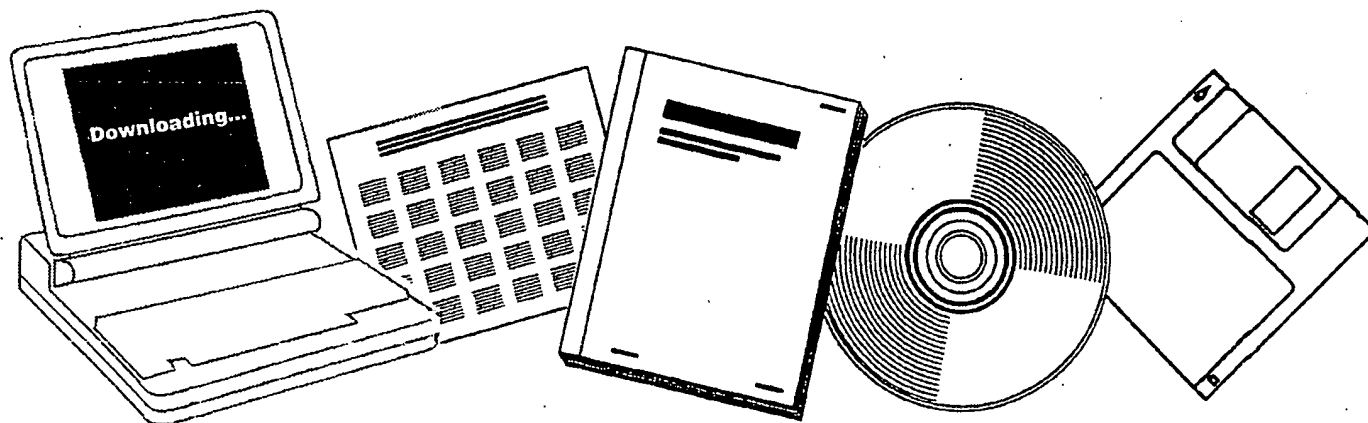
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**CHEMICAL AND ENZYMATIC TRIGGERING OF  
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CHEMILUMINESCENCE FROM  
TERT-BUTYLDIMETHYLSILYLOXY-SUBSTITUTED  
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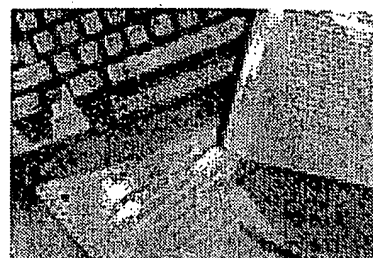
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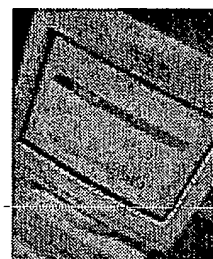
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TECHNICAL REPORT #3

CHEMICAL AND ENZYMATIC TRIGGERING OF 1,2-DIOXETANES 2:  
 FLUORIDE-INDUCED CHEMILUMINESCENCE FROM  
 TERT-BUTYLDIMETHYLSILYLOXY-SUBSTITUTED DIOXETANES

by

A. Paul Schaap, Tsae-Shyan Chen, Richard Handley, Renuka DeSilva, and B. P. Giri

Prepared for Publication

in

Tetrahedron Letters

Department of Chemistry  
 Wayne State University  
 Detroit, MI 48202

March 17, 1987

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CHEMICAL AND ENZYMATIC TRIGGERING OF 1,2-DIOXETANES. 2:  
FLUORIDE-INDUCED CHEMILUMINESCENCE FROM  
TERT-BUTYLDIMETHYLSILOXY-SUBSTITUTED DIOXETANES

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**Abstract:** Thermally stable 1,2-dioxetanes bearing *tert*-butyldimethylsilyloxyaryl groups have been prepared. Reaction of these dioxetanes with fluoride ion at ambient temperature in MeCN and DMSO generates chemiluminescence with efficiencies up to 25%.

In 1982 we demonstrated that chemiluminescence from a 1,2-dioxetane bearing a phenolic substituent could be triggered by the addition of base.<sup>1</sup> Deprotonation generates an unstable phenoxide-substituted dioxetane which decomposes  $4.4 \times 10^6$  times faster than the protonated form. We have now used this initial observation to develop other methods for triggering the chemiluminescent decomposition of thermally stable dioxetanes. For example, we have recently shown that a naphthyl acetate-substituted dioxetane can be *enzymatically* cleaved in aqueous buffers using aryl esterase.<sup>2</sup> We now provide the first example of chemical triggering of silyloxy-substituted dioxetanes by fluoride in DMSO or MeCN to generate chemiluminescence with efficiencies up to 25%.

Dioxetanes **2a-c** were prepared by photooxygenation of the corresponding alkenes<sup>3</sup> in  $\text{CH}_2\text{Cl}_2$  using polymer-bound Rose Bengal<sup>4</sup> (SENSITOX I) and a 1000-W high-pressure sodium lamp. After 15 - 30 min irradiation the sensitizer was removed by filtration and the solvent evaporated under vacuum. Recrystallization of the material from pentane or chromatography over silica gave the dioxetanes.<sup>5</sup> Rate constants for the thermal decomposition of **2a-c** were obtained at 80 to 120 °C from measurements of the decay of chemiluminescence intensity of  $10^{-4}$  M solutions in *o*-xylene.<sup>6</sup> Chemiluminescence spectra from **2a-c** all exhibited a maximum at 437 nm indicating that the luminescence is derived from singlet excited adamantanone and not the phenyl or naphthyl esters. Rates showed variations of less than 3% and gave excellent Arrhenius plots ( $r > 0.99$ ) with activation energies for **2a-c** of 29.7, 27.0, and 28.4 ( $\pm 1$ ) kcal/mol, respectively (Table 1). Half-lives for **2a-c** at 25 °C are calculated to be several years.<sup>7</sup> These results demonstrate the high degree of stabilization that can be obtained with sterically hindered adamantyl-substituted dioxetanes.<sup>8</sup>

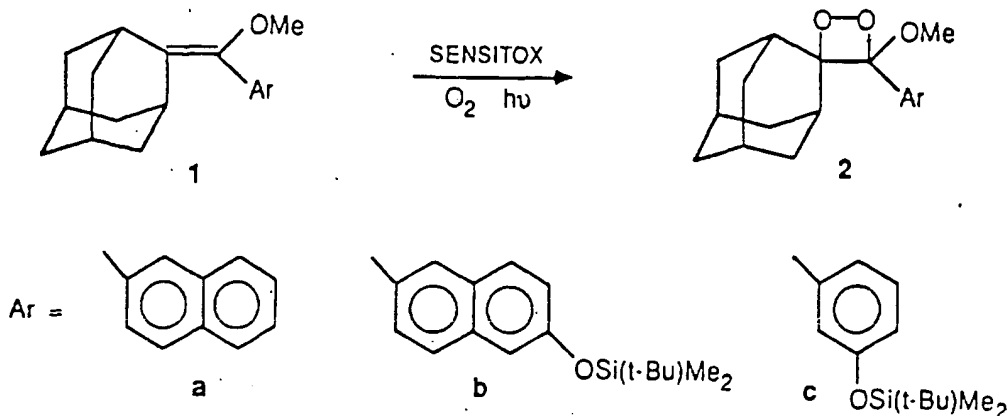


Table 1. Activation Parameters and Rates of Decomposition for 1,2-Dioxetanes 2a-c in *o*-Xylene.

dioxetane	$E_a$ (kcal/mol)	log A	$k(\text{sec}^{-1})$ at 25 °C	half-life at 25 °C <sup>a</sup>
2a	29.7	13.2	$3.17 \times 10^{-9}$	6.9 years
2b	27.0	11.7	$8.72 \times 10^{-9}$	2.5 years
2c	28.4	12.6	$5.74 \times 10^{-9}$	3.8 years

(a) Calculated from the Arrhenius plots.

Deprotection of silyl ethers with fluoride is a widely used reaction in modern organic synthesis.<sup>9</sup> We have now used this procedure to generate the unstable, chemiluminescent aryloxide dioxetanes **3b** and **3c** from the thermally stable forms **2b** and **2c**, respectively. In a typical experiment injecting an aliquot of an *o*-xylene solution of dioxetane **2b** into 3 mL of 0.001 M *n*-Bu<sub>4</sub>NF in MeCN to give a final dioxetane concentration of 10<sup>-5</sup> M produced a flash of blue chemiluminescence which decayed by a pseudo-first-order process with a half-life of less than 1 sec at room temperature. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm which was identical to the fluorescence of **4b**<sup>10</sup> and the fluorescence of the spent dioxetane solution under these conditions. No chemiluminescence derived from adamantanone fluorescence appears to be produced. Analysis of the crude product mixture resulting from treatment of a sample of **2b** with 1 equivalent of *n*-Bu<sub>4</sub>NF by <sup>1</sup>H NMR and UV revealed only the expected cleavage products: adamantanone and the tetra-*n*-butylammonium salt of methyl 6-hydroxy-2-naphthoate in a 1:1 ratio.

The chemiluminescence quantum yield for the fluoride-triggered decomposition of dioxetane **2b** was measured relative to the luminol standard<sup>11</sup> using a photon-counting apparatus.<sup>12</sup> Fluoride-triggered decomposition of 10<sup>-5</sup> M solutions of **2b** in MeCN at 25 °C produced chemiluminescence with a quantum yield of 4 x 10<sup>-5</sup> which was independent of fluoride concentration in the range 10<sup>-2</sup> to 10<sup>-4</sup> M. Correction for the fluorescence quantum yield of **4b** in MeCN under identical conditions leads to a calculated chemiexcitation quantum yield of 1.1 x 10<sup>-4</sup> or an efficiency for the formation of singlet excited **4b** of 0.01% (Table 2).<sup>13</sup>

In contrast to the low chemiluminescence quantum yield on triggering the decomposition of **2b**, we find that treatment of **2c** with fluoride produces chemiluminescence with dramatically higher efficiency. Addition of excess *n*-Bu<sub>4</sub>NF to 10<sup>-7</sup> M solutions of dioxetane **2c** in MeCN resulted in a rapid decomposition of **2c** accompanied by bright blue chemiluminescence with a half-life of 5 sec at 25 °C. The pseudo-first-order rate constant was independent of fluoride concentration in the range 6.7 x 10<sup>-5</sup> to 3.3 x 10<sup>-3</sup> M. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm in MeCN which matched exactly the fluorescence of **4c** under these conditions. Similar experiments were conducted in DMSO (Figure 1).<sup>14</sup> Quantum yields for the chemiluminescence of **2c** with

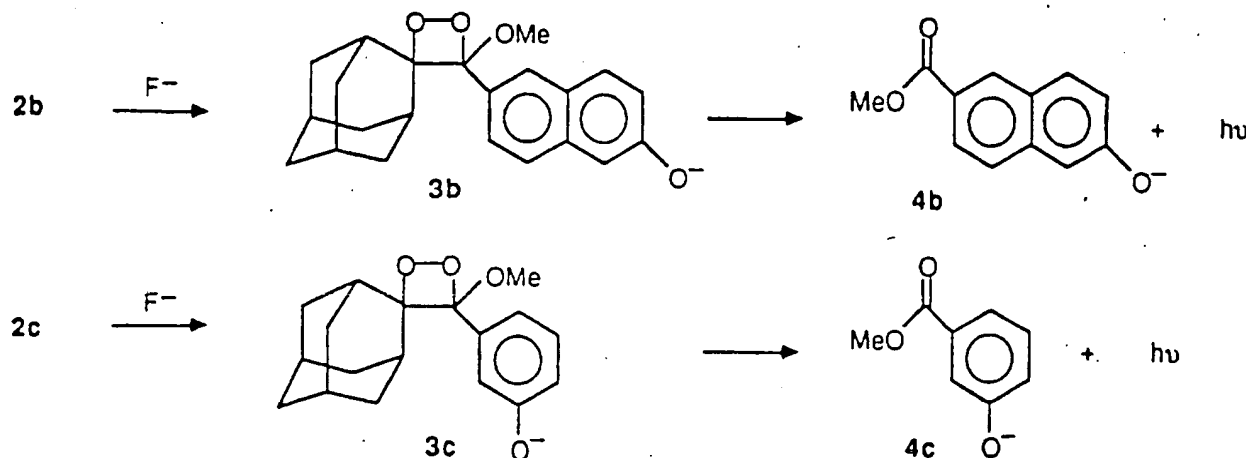


Table 2. Fluoride-Induced Chemiluminescence from Dioxetanes 2b and 2c.

dioxetane	solvent	$\Phi_{\text{CL}}^{\text{a}}$	$\Phi_{\text{F}}^{\text{b}}$	$\Phi_{\text{CE}}^{\text{c}}$
2b	MeCN	$4 \times 10^{-5}$	0.37	$1.1 \times 10^{-4}$
2c	MeCN	0.094	0.21	0.45
2c	DMSO	0.25	0.44	0.57

(a). Chemiluminescence quantum yields. (b). Fluorescence quantum yields for cleavage products 4b and 4c relative to quinine sulfate with a value of 0.54.

(c). Quantum yields for the formation of the singlet excited state of 4b and 4c.

fluoride were determined in MeCN and DMSO<sup>15</sup> relative to the luminol standard and found to be 0.094 and 0.25, respectively (Table 2). Correction for the fluorescence quantum yields of 4c in these solvents gives efficiencies for the formation of singlet excited 4c of 45 and 57%, one of the highest singlet chemiexcitation efficiencies yet reported for a dioxetane.<sup>16</sup>

These results are readily explained by a mechanism initiated by cleavage of the Si-O bond by fluoride to generate the unstable dioxetanes 3b and 3c. The lack of any dependence of the rate of decay of the chemiluminescence on fluoride concentration suggests that the rate-limiting step under these conditions may be the cleavage of the aryloxy dioxetanes 3b and 3c.<sup>17</sup> The rapid decomposition of these intermediates is induced by an intramolecular electron transfer from the strongly electron-donating phenoxide type substituent to the peroxide  $\sigma^*$  orbital.<sup>1</sup> Similar mechanisms have been proposed for the efficient chemiluminescence from dioxetanes bearing easily oxidized substituents.<sup>18</sup> Chemiluminescence has also been observed from intermolecular electron-transfer reactions between peroxides and fluorescent hydrocarbons.<sup>19</sup> The reason for the significant difference in the chemiexcitation efficiencies of 3b and 3c is currently under investigation. We have prepared the corresponding *p*-tert-butyltrimethylsilyloxyphenyl-substituted dioxetane and found the chemiexcitation efficiency to also be less than 0.01% in that case.

We are continuing our work with dioxetane 2c and related derivatives with a view towards the possible use of this system as a convenient liquid light standard. Solutions of 2c prepared in *o*-xylene exhibit high stability and can be stored for long periods. A stock solution of 2c kept at room temperature gave identical results after one month. Typically, the calibration of a luminometer can be carried out by injecting 30  $\mu\text{L}$  of a  $10^{-5}$  M solution of 2c into 3 mL of a 0.003 M solution of *n*-Bu<sub>4</sub>NF in dry DMSO. The luminescence is emitted over a 20 sec period with 1-2% reproducibility for the total light emission.

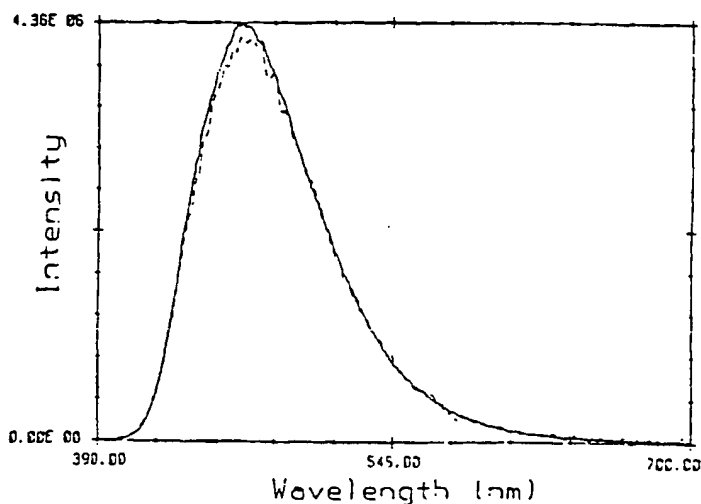


Figure 1. Chemiluminescence spectrum from fluoride triggering of dioxetane 2c in DMSO (---). Fluorescence spectrum of 4c under the same conditions (—).



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**Acknowledgments.** Support of this research by the Office of Naval Research is gratefully acknowledged. We also wish to thank Mr. Marc Budrick for his assistance with the synthesis of the alkenes.

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2. Schaap, A. P.; Handley, R. S.; Giri, B. P. *J. Am. Chem. Soc.*, submitted for publication.
3. The preparation of the alkenes will be described in a subsequent paper.
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5. 4-Methoxy-4-(2-naphthyl)spiro[1,2-dioxetane-3,2'-adamantane] (2a): mp 116 °C (dec); <sup>1</sup>H NMR δ 0.9-2.0 (m, 12H), 2.22 (s, 1H), 3.11 (s, 1H), 3.24 (s, 3H), 7.0-8.3 (m, 7H); <sup>13</sup>C NMR δ 25.94, 26.07, 31.60, 31.72, 32.31, 33.08, 33.23, 34.88, 36.42, 50.00, 95.60, 112.33, 125.21, 126.47, 127.02, 127.63, 127.91, 128.67, 129.41, 132.13, 132.85, 133.61. 4-(6-*tert*-Butyldimethylsilyloxy-2-naphthyl)-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane] (2b): mp 107 °C (dec); <sup>1</sup>H NMR δ 0.27 (s, 6H), 1.03 (s, 9H), 1.4-2.0 (m, 12H), 2.2 (s, 1H), 3.1 (s, 1H), 3.23 (s, 3H), 7.1-7.85 (m, 6H); <sup>13</sup>C NMR δ -4.33, 18.23, 25.67, 25.93, 26.06, 31.59, 31.69, 32.31, 33.04, 33.19, 34.86, 36.42, 49.94, 95.59, 112.44, 114.63, 122.58, 126.64, 128.50, 129.85, 130.11, 134.93, 154.59. 4-(3-*tert*-Butyldimethylsilyloxyphenyl)-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane] (2c): oil, <sup>1</sup>H NMR δ 0.20 (s, 6H), 0.99 (s, 9H), 1.26-1.90 (m, 13H), 3.02 (s, 1H), 3.23 (s, 3H), 6.86-7.30 (m, 4H); <sup>13</sup>C NMR δ -4.34, 18.33, 25.77, 26.18, 26.07, 31.62, 31.70, 32.50, 33.00, 33.26, 34.80, 36.56, 49.94, 95.30, 111.91, 119.32, 121.26, 123.90, 129.18, 136.10, 155.86.
6. NMR experiments have shown that 2b-c undergo thermal decomposition to give 2-adamantanone and the expected silyloxyaryl ester.
7. Actual half-lives could be shorter if the solvents used for storage of the dioxetanes contained impurities which could lead to catalytic decomposition. Experiments are in progress to provide a direct measure of the stability of these dioxetanes at ambient temperature.
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10. Solutions of 4b and 4c were produced by treating the silyloxy-protected esters with fluoride.
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15. Spectral grade MeCN was obtained from Burdick and Jackson Laboratories, Inc. Reagent grade DMSO from several sources gave identical quantum yields. We have used the trihydrate of *n*-Bu<sub>4</sub>NF for these experiments. Other sources of fluoride produced similar results. We have noted that direct addition of fluoride to "old" samples of these solvents produces a very weak chemiluminescence in the absence of the dioxetanes.
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